chain) made of either pure PC or a 3:1 mixture with a charged phospholipid (PG). To further identify and characterize their effect on channel activity, we measured simultaneously ionic current and position of single ion channels using a bilayer voltage-clamp system combined to a single-molecule fluorescence microscope. In all recordings containing several channels, clustering appeared to be important. Direct clustering events of freely diffusing single KcsA channels were observed in bilayers of various thicknesses, while channel activity remained constant. However, channel function appeared to be modulated by the bilayer thickness. Interestingly, despite a negative phospholipid headgroup has been previously suggested as an essential component for KcsA function, channel activity was still recorded without PG for bilayers close to physiological membrane thickness (~27 Å), suggesting that mechanical stress may compensate for the absence of the negatively charged head group. Moreover, clustering could also have a regulating effect since appearance of coupled channel activity showing significantly higher conductance than what is expected for KcsA was observed in the presence of clusters.

3736-Pos Board B464

Molecular Characterization of the Binding of Polyunsaturated Fatty Acids to a Voltage-Gated Potassium Channel

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In refractory epilepsy, a ketogenic diet (ample protein and low carbohydrate intake) has proven to be a very effective treatment. Fatty acids, and in particular polyunsaturated fatty acids (PUFAs) have been established as the key constituents in the anticonvulsant property of this diet. Even though this diet has been used since the 1920s, the underlying mechanisms by which it operates and prevents the epileptic seizures have been an eluding factor to this day. PUFAs are key players in the regulation of neuronal excitability by controlling sodium and calcium currents, albeit little is known of their effects on voltage-gated K channels. A reduced K^+ current, caused by mutations in Kv1-type and KQT-type voltage-gated K^+ channels, has been observed to cause epilepsy. Accordingly, an increased K^+ current sparked by PUFAs could act as an antiseizure mechanism. There is some experimental evidence that PUFAs act on the voltage sensor domain by binding to the lipid bilayer and attracting the positive charges of the voltage sensor to the extracellular side of the channel, i.e. the open configuration, thereby leading to an increased K^+ current. Here, we report on studies of this process using molecular simulations that show how PUFA-enriched lipid bilayers interact with an integral voltage-gated ion channel, their enrichment on specific regions on the voltage sensor, and how this might help explain the selective stabilization of the open state of a voltage-gated K^+ channel.

3737-Pos Board B465

A Non-Canonical Di-Acidic Signal at the C-Terminal of KV1.3 Determines Anterograde Trafficking and Surface Expression

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Impairment of Kv1.3 membrane expression in leukocytes and sensory neuron contributes to the pathophysiology of autoimmune diseases and sensory syndromes. Molecular mechanisms underlying Kv1.3 channel trafficking to the plasma membrane remain elusive. We report a novel non-canonical di-acidic signal ($E^{483/484}$) at the C-terminus of Kv1.3 essential for anterograde transport and surface expression. Notably, homologous motifs are conserved in neuronal Kv1 and Shaker channels. Biochemical analysis reveals interactions with the Sec24 subunit of the coat protein complex II. Disruption of this complex drastically retains the channel at the endoplasmic reticulum. A molecular model of the Kv1.3-Sec24a complex suggests salt-bridges between the di-acidic $E^{483/484}$ motif in Kv1.3 and the di-basic $R^{750/752}$ sequence in Sec24. These findings identify a novel and previously unrecognized motif of Kv channels essential for their expression in the cell surface. Our results contribute to our understanding of how Kv1 channels target to the cell membrane, and provide new therapeutic strategies for the treatment of pathological conditions.

3738-Pos Board B466

Kv1.3-Blocking Peptides from Parasitic Worms Exhibit Immunomodulatory Function

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Parasites have coexisted with their human hosts for thousands of years and are known mainly for their harmful disease-causing role in humans. However, probiotic worm therapy is beneficial in human autoimmune diseases, suggesting the existence of immunomodulators in parasitic worms. By screening a cDNA library and searching genome databases we identified a family of peptides in parasitic worms that share sequence similarity and evolutionary relatedness to potassium channel-blocking peptides secreted by venomous sea anemones. AcK1, a 51-residue secreted peptide of the hookworm *Ancylostoma caninum,* and BmK1, the C-terminal domain of a 413-residue zinc metalloprotease from the filarial worm *Brugia malayi*, share structural similarity to ShK and BgK peptides from sea anemones. These peptides block cloned and native human T-cell Kv1.3 channels at nanomolar to low micromolar concentrations. BmK2, an analog of BmK1, blocks Kv1.3 with an IC_{50} of 2 nM and exhibits >4000-fold selectivity for Kv1.3 over Kv1.1, Kv1.2, Kv1.6, Kv3.2, KCa3.1, K_{2P}3.1. These peptides suppress proliferation of effector memory T cells that use Kv1.3 channels to regulate membrane potential, without affecting other T cell subsets that are not dependent on Kv1.3. They inhibit cytokine production and suppress the in vivo delayed type hypersensitivity response. Our results provide a mechanistic basis for pro-biotic worm therapy in human autoimmune disease, and suggest that these or related peptides and proteins could supplant the need for worm therapy.

3739-Pos Board B467

Biophysical Characterization of the Potassium Channel Kv1.3 in B Cells from Patients Affected by Chronic Lymphocytic Leukemia

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Recent discoveries identified ion channels as possible targets for cancer treatment. We have previously demonstrated that inhibition of the mitochondria-located potassium channel Kv1.3 (mtKv1.3) by membrane permeant inhibitors Psora-4, PAP-1 and clofazimine triggers cell death both in vitro and in vivo, resulting in reduction of tumor volume up to 90% (Leanza et al, 2012, EMBO Molecular Medicine). Importantly, these compounds are able to selectively kill pathological B cells from patients affected by chronic lymphocytic leukemia (B-CLL) (Leanza et al, 2013, Leukemia). Here, we determined the expression profile of Kv1.3 by western blot comparing B cells from healthy subjects and B-CLL patients in whole cell lysates as well as in purified mitochondria. An increased expression of the channel in pathological cells compared to normal ones was observed. To our knowledge, electrophysiological characterization of Kv1.3 in B-CLL cells has not been performed up to now. By measuring Kv1.3 current in patch clamp experiments, both at whole cell and at single channel levels, here we show that increased protein expression was correlated with enhanced channel activity. Biophysical and pharmacological properties of Kv1.3 from the two cells types are reported. Experiments with

pathological B-cells cultured in the presence of cells mimicking bone marrow environment are in progress.

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The Sh3-Binding Domain of Kv1.3 Channels is Required for their Cortactin-Conveyed Coupling to Actin

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Kv1.3 channels of T lymphocytes play an important role in several physiological functions like activation or migration, which are accompanied by the redistribution of channels in the cell membrane. Both processes are characterized by F-actin polymerization indicating the involvement of the actin cytoskeleton in the associated channel's redistribution. Several studies reported that Kv1.x channels are linked to or co-localize with various adapter proteins which also possess actin-binding domains: hDlg1 via the PDZ-binding domain and cortactin through either the cortactin- or the SH3-binding site. In this study we investigated the molecular requirements for Kv1.3 channel lateral membrane diffusion.

FLAG/EGFP-tagged Kv1.3 wild-type or mutant constructs were expressed in HEK-293 cells. We designed C-terminal truncated (without (delta1) or with (delta2) cortactin binding sequence, and both without SH3; with cortactin and SH3 binding motif (delta3)) and few-amino-acid Kv1.3 mutants (deltaSH3/deltaPDZ: SH3/PDZ domain ruptured). Biophysical properties of each phenotype were evaluated with patch-clamping. Interaction/co-localization between channel and adaptor proteins was studied with co-immunoprecipitation/confocal microscopy. Lateral mobility of Kv1.3 was assessed with FRAP and defined by the channel's mobile fraction (M_f).

Parameters of channel gating for mutants were indistinguishable from those of wild-type Kv1.3. Confocal microscopy images and co-immunoprecipitation experiments demonstrated that cortactin and Kv1.3 interact in HEK-293 cells. Furthermore, wild-type, delta3 and deltaPDZ mutants have reduced M_f upon stimulation of F-actin polymerization/stabilization by jasplakinolide (unlike delta1, delta2 and deltaSH3 constructs). When cortactin was knocked down by shRNA, exposure to jasplakinolide induced no decrease in M_f of wild-type Kv1.3 channels. These findings point out that cortactin serves as a bridge between Kv1.3 and the actin meshwork via the channel's SH3-binding domain, and can regulate mobility/immobility of Kv1.3 channels. (NIH 2R01CA095286).

3741-Pos Board B469

Role of Kv1.3 Potassium Channels in Auditory Function

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Kv1.3 is a low threshold voltage-dependent potassium channel involved in various physiological functions. Within the central nervous system, deletion of Kv1.3 gene from mitral cells of the olfactory bulb dramatically increased the sensitivity of the olfactory system. We have recently shown that Kv1.3 channels are present in presynaptic terminals of the medial nucleus trapezoid body (MNTB) within the auditory brainstem and in bouton-like structures on inner and outer hair cells within the cochlea. Whether Kv1.3 channels contribute to auditory function is, however, unknown. We have therefore used in vivo and in vitro approaches to examine the role of Kv1.3 channels in the peripheral and central auditory system. Auditory brainstem responses (ABR) show that the thresholds of ABR are elevated in 2-4 months old Kv1.3-/- KO mice over those in wild type (WT) mice. Latencies of peaks I, II and IV are prolonged in 4 month old Kv1.3 -/- KO mice. In addition, we have found a desynchronization of ABR waves in Kv1.3-/- KO mice suggesting an alteration of synaptic transmission and changes in spike fidelity within auditory pathways. To further investigate the mechanisms of these alterations in ABR waves in Kv1.3-/- KO mice, we carried out in vitro slice recordings of the high fidelity calyx of Held/MNTB synapse. Our preliminary results from whole cell patch-clamp recordings in young mice (P13-17) show that lack of Kv1.3 channels increases the spike frequency and the spike threshold at presynaptic terminals (Calyx of Held) in response to square pulses of injected currents. Our preliminary data showing that loss of Kv1.3 channels primarily influences the properties of presynaptic terminals and of the ABR waves strongly suggest that Kv1.3 channels are required for normal auditory function.

3742-Pos Board B470

Exploring the Effect of Gambierol on the Gating Machinery of Kv3.1 Channels

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Gambierol is a ladder-shaped polyether toxin that acts as a gating modifier to inhibit Kv3.1 channels with nanomolar affinity. Binding determinants for gambierol have been identified at an interface between S5 and S6, located outside the permeation pathway. However, the high gambierol sensitivity of Kv3.1 channels could not be fully transplanted to the insensitive Kv2.1 channel by introducing the S5-S6 determinants. To explore whether also the voltage-sensing domain (VSD) is a determinant for gambierol sensitivity, we exchanged the complete VSD (S1-S4), parts of the VSD (the S1-S3a region and the Sb-S4 paddle), and the electromechanical coupling (L45+S6c) between Kv3.1 and Kv2.1. Our results show that the L45+S6c and the S1-S3a region did not alter the affinity of Kv3.1 channels for gambierol. In contrast, the distal part of the VSD, the S3b-S4 paddle, displayed a 100-fold decrease in affinity compared to WT Kv3.1. Since all VSD chimeras displayed similar biophysical properties and remained sensitive to well-known pore blockers, the loss in gambierol sensitivity in the S3b-S4 paddle chimera is most likely not the result of allosteric effects. Molecular-Dynamics simulations indicated that the S3b-S4 paddle motif resides in proximity of gambierol and that the structure and position of the VSD may regulate the space of the binding site between the pore domain (S5 and S6) and the gating machinery. Hence, our results suggest that the VSD, and especially the S3b-S4 paddle motif, contributes to the structure and/or the accessibility of the binding site for gambierol. (This research was supported by FWO grant G0433.12N to DJS and JT).

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Regulation of K_V1.5 Channel Density in the Rat Atria

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The density of functional Kv1.5 channels underlying I_{Kur}, the main repolarizing current in human atria, is a result of the equilibrium between exocytosis and endocytosis. We have shown that in addition to constitutive exocytosis, Kv1.5 channels can also undergo regulated exocytosis, for instance following changes in the mechanical environment or changes in the cholesterol content of the sarcolemma. Although constitutive and triggered endocytosis have been investigated for Kv1.5, its internalization pathway has not been described. Using high-resolution 3-D deconvolution microscopy, we showed that Kv1.5 channels are associated with clathrin vesicles (CVs) in atrial myocytes. Electron microscopy (EM) showed that CVs are found both at the intercalated disc and at the lateral sarcolemma, aligned along z-bands. Blockade of the clathrin pathway using hypertonic media or SiRNA increased I_{Kur} densityin atrial myocytes and led to Kv1.5 channels accumulating at the sarcolemma, as shown by biotinylation assays and fluorescence recovery after photobleaching (FRAP) experiments. These data support the hypothesis that Kv1.5 channels are internalized *via* the clathrin pathway.

Next, we investigated Kv1.5 channel internalization in a rat model of atrial hemodynamic overload. Despite reduced Kv1.5 protein expression in dilated atria, I_{Kur} density was unchanged, suggesting increased functional Kv1.5 channels at the sarcolemma. Clathrin expression was reduced in dilated atria, and a decreased colocalization between Kv1.5 channels and CVs was observed. However, EM showed no significant difference in internalization activity between sham and dilated atria. Therefore, the reduced clathrin protein synthesis observed in dilated atria is not likely to be responsible for the accumulation of Kv1.5 channels at the sarcolemma. Other mechanisms such as increased recycling and/or membrane stabilization must be investigated to understand how I_{Kur} is maintained in dilated atria.

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Hammond Energy Shifts Reveal Sequence of Conformational Changes in N- and C-Type Inactivation of Kv1.4

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C-type inactivation is sensitive to mutation on extra- and intracellular side of the channel, indicating it may involve conformational changes at both